European Patent Office
Erhardstrasse 27
8000 München 2
Germany

Re: Opposition to European Patent EP 0 125 023 (Genentech, Inc.)

AFFIDAVIT OF HERBERT L. HEYNEKER

I, Herbert L. Heyneker, declare and state as follows:

1. I am an inventor of the above-identified patent.

2. I have more than twenty years of professional experience doing research in the field of recombinant DNA technology. In 1975, I completed research required for and obtained a degree of Doctor of Philosophy in Molecular Genetics from the University of Leiden, the Netherlands. From 1975 to 1977, I did post-doctoral research as a fellow at the University of California, San Francisco School of Medicine, where my advisor was Professor Dr. Herbert W. Boyer. I was among the first scientists who joined Genentech, Inc., in 1978. In the following six years I retained employment with Genentech as a Senior Scientist, and was involved in virtually every Genentech molecular biology product, including, in addition to our work with antibodies, the cloning and expression of human insulin, human proinsulin, human growth hormone, bovine and porcine growth hormones, human leukocyte interferon,

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human urokinase, human tissue plasminogen activator (ht-PA). Since my departure from Genentech, I have founded three biotech companies. At present, I am founder and CEO of Array Technologies, Inc., Palo Alto, California. A copy of my Curriculum Vitae, including a list of my publications, patents and other professional activities, is attached to and forms part of this Declaration (Exhibit "A").

3. In 1980, Dr. Riggs spent a few months at Genentech on sabbatical from City of Hope, with the intention of investigating the possibility of producing antibodies in bacteria. The next year, following a proposal by Dr. Riggs, we started working on the antibody project. Our goals included the cotransformation of *E. coli* with both the heavy and light chain genes of an antibody to the carcinoembyonic antigen (CEA). CEA is associated with the surface of certain human tumor cells. Antibodies directed to CEA were therefore thought to be useful in detecting, and potentially useful in treating human tumor cells which express CEA at their surfaces. Thus, anti-CEA antibodies were a model system for investigating and expressing antibody genes. Dr. Shmuel Cabilly, who was then a Post Doctoral Fellow at City of Hope, used a hybridoma cell line called "CEA.66.E3" (obtained from Dr. John Shively) to extract total RNA, and purify mRNA, as described in our patent. While our work was performed with anti-CEA antibodies, in 1983 it would have been easy to produce a murine hybridoma secreting another antibody capable of binding a given antigen. Since the hybridoma by definition would be secreting only one immunoglobulin (in addition to the immunoglobulin, if any, secreted by the parental fusion partner), it would have been straight-forward to prepare cDNAs from mRNA of this hybridoma that encode the desired heavy and light chains. Identification of *E. coli* transformants containing a cDNA insert in the cloning vector coding for the desired heavy and light chains could have been accomplished, for example, by using as probes DNA encoding portions of the constant domains of the murine immunoglobulin heavy and light chains, substantially as shown in Examples E.1.1 to E.1.6 of the specification with respect to an anti-CEA antibody.

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4. In the early 1980's, *E. coli* was the work horse of heterologous protein production by recombinant DNA technology. Therefore, most of our work at Genentech focused on heterologous protein expression in bacteria, and in particular, *E. coli*. This is why the City of Hope scientists and our group chose this organism as a model system in our endeavors to demonstrate the feasibility of the production of antibodies, including chimeric antibodies, in genetically engineered recombinant host cells. Proceeding as described in the above-identified European patent, we were able to express both the anti-CEA antibody heavy and light chains in a single *E. coli* host cell. Dr. Cabilly used the vector pKCEAtrp207-1*delta to transform *E. coli* conferring resistance to tetracycline, and then retransformed these *E. coli* with pγCEAInt2 thereby conferring resistance to ampicillin. These cotransformed cells could be identified by their resistance to both tetracycline and ampicillin. Detailed of this work are provided on page 10 of the above-identified patent. The cotransformed cells were treated with indoleacrylic acid (IAA) to induce the TRP promoter in order to make high levels of light chain and heavy chain in the form of refractive bodies. An SDS-PAGE gel showed that the cotransformants were expressing both heavy and light chains (page 19, lines 18-22, and lines 31-33). It was clear to us that the purification and refolding of anti-CEA antibody chains from singly and doubly transformed cells was important to the success of our work. Antibody chains were purified by lysing frozen bacterial cells by sonication, followed by centrifugation to obtain the refractile bodies. It was found that heavy and light chains could be isolated from both single and cotransformed bacteria by guanidine solubilization of the refractile body preparations, without any further purification by column chromatography (see, e.g. page 20, lines 47-48). These samples could then be used directly in refolding reactions. The final conditions involved a mixture of sulfitolized heavy chain and an extract of light chain cells. Under preferred conditions, the chains were reconstituted by incubation in β-mercaptoethanol as a reducing agent in the presence of EDTA, at a pH of 8.5 in concentrated urea, followed by dialysis in a nitrogen saturated buffer consisting of 0.5M urea in 0.1M sodium glycinate, pH 10.8, 1mM EDTA, 5mM reduced glutathione, 0.1mM oxidized glutathione and 10 mM glycine ethyl ester at 4°C. These conditions are

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clearly set forth at page 20, lines 47-54 of our patent. In one of our experiments, CEA binding activity was regenerated after refolding. The results set forth in the table on page 21 show a refolding yield of 0.76% starting from a cotransformed cellular extract, and a yield of 0.33% starting from a mixture of a heavy chain S-sulfonate and the urea solubilized crude extract of light chain producing cells. The value of 1580 ng/ml in the cotransformed refolding reaction was significantly higher than the background levels of apparent activity obtained from controls of either heavy chain alone (441 ng/ml - not shown in the table) or light chain alone (108 ng/ml); these latter values arise from non-specific binding to CEA in the assay. This data shows that heavy chain and light chain recombine in the refolding reaction to generate antigen binding activity.

5. While the examples of the above-identified patent concern the use of *E. coli* as a host organism, and the actual data show the accumulation of the antibody produced in inclusion bodies, it was always clear to us that other host organisms would be similarly suitable, and that the recovery of the antibodies could be simplified and the yields increased by means which were available and well known to molecular biologists at the priority date of this patent (April 8, 1983). Thus, we knew that tissue cultures of multicellular organisms were suitable expression hosts. This is clearly expressed on page 9, lines 5-28 of our European patent. Means and methods for propagation of vertebrate cells in cell culture were known as early as 1973 (Tissue Culture, Academic Press, Kruse and Patterson, eds., (1973) - cited on page 9 of the patent). A large variety of permanent cell lines were available, prepared by successive serial transfers from isolate normal cells. For example, a CV-1 monolayer was described by Mertz *et al.*, Virology 62:112 (1974) (Exhibit "C"). The COS-7 line of monkey cells were described by Gluzman, Cell 23:175 (1981) (Exhibit "D"). Other cell lines, e.g. WI38, BHK, 3T3, CHO, VERO and HeLa cell lines were similarly available. For example, CHO-K1 cells could be obtained from the American Type Culture Collection (ATCC CCL61). McClutchan and Pagano (J. Natl. Cancer Inst. 41:351 (1968) - Exhibit "E") disclosed a DEAE-dextran procedure for introducing DNA into

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mammalian cells. Graham and Van der Eb (Virology 52:456 (1973) - Exhibit "F") described calcium phosphate transfection to introduce adenovirus DNA into mammalian cells. This reference is cited at page 9, line 35 of the specification. Subsequently, this technique was found to be suitable for integrating exogenous DNA into mammalian cells (Wigler *et al.*, Cell 14:725 (1978) - Exhibit "G"). The construction of expression vectors, including the selection of suitable promoters is described on page 9 of our patent. As stated at page 9, line 18, the complete nucleotide sequence of Simian virus 40 (SV40) DNA was published by Fiers *et al.*, Nature 273:113 (1978) (Exhibit "H"). The cited paragraph also describes how to use this virus to provide the control functions for mammalian expression vectors. Indeed, the construction of SV40-based expression vectors capable of replicating in various vertebrate cell culture systems, and the production of heterologous polypeptides in such vertebrate host culture systems by recombinant DNA technology were disclosed in Genentech's European Patent Application Publication No. 0 073 656, which was published on March 9, 1983, and thus was publicly available at the priority date of the above-identified patent. A copy of this publication is attached as Exhibit "I". The latter application specifically describes the production of hepatitis B surface antigen (HBsAg) in COS cells. Based upon the experience with HBsAG and human tissue plasminogen activator (ht-PA) (EP 0 093 619 - Exhibit "J"), our first choice for a mammalian host would have been a COS cell line, which is preferred for its ease of use. As a next step, we would have scaled up the antibody production in a stable CHO cell line. This cell lines could have been transformed, for example, with an expression vector containing the Simian Virus 40 (SV 40) early and late promoters, along with the SV40 viral origin of replication, as described on page 9, lines 16-26 of the present European patent, and also in EP 0 073 656. This work could have been done without excessive experimentation.

6. We also planned the expansion of our *E. coli* work by fusing *E. coli* signal sequences to the N-terminus of the antibody chains. Our first choice would have been the h.s.t. II signal (Lee, C.H., Infect. Immun. 42:254-268 (1983); Picken, R.N., Infect. Immun. 42:269-275 (1983) - Exhibits "K" and

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"L", both published shortly after our priority date), which was successfully used in our work with ht-PA. This signal would have been reasonably expected to secrete the antibody chains at least into the periplasmic space of *E. coli*, thereby facilitating their recovery. I would have expected, for example, the known OmpA signal sequence to work in a similar way.

7. Prior to our work, monoclonal antibodies were produced by hybridomas, which have the intrinsic limitation that they can only produce native-sequence antibodies as they occur in the B-cell population. By completing the work disclosed in the above-identified patent and in our subsequent scientific paper (Cabilly *et al.*, Proc. Natl. Sci. USA 81:3273-3277 (1984) - Exhibit "B"), we demonstrated that antibodies, including chimeric antibodies, can be produced by recombinant DNA technology. This opened the way to a wide range of manipulations of the native antibody sequence and structure, and made possible the design of antibody structures which have the advantage of being less immunogenic than native rodent antibodies when used as human *in vivo* diagnostics or therapeutics.

8. Unhappily, at 1983 Genentech was a small company with limited resources. Human growth hormone, human tissue plasminogen activator, insulin, and hepatitis B antigen were identified as potential products, and received priority. Due to economic considerations, our antibody work could not been given the support it would have required and that we were hoping for. It is for this reason that we did not generate more experimental data following the filing of the U.S. application on which the present European patent is based, and not because we failed to recognize the possibilities for improvement, or believed that the present invention could not have been successfully carried out by a variety of other methods. My personal disappointment over the fate of the antibody project was instrumental in my leaving the company in 1984.

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9. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Herbert L. Heyneker, Ph.D.

Date: March 20, 1997